within months. This tumor needs to be recognized and separated from the more common poorly differentiated ovarian adenocarcinoma because of its poor prognosis, association with hypercalcemia, occurrence in young women and resistance to all current modes of therapy.

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REFERENCES

Dickersin GR, Kline IW, Scully RE: Small cell carcinoma of the ovary with hypercalcemia: A report of eleven cases. Cancer 1982 Jan; 49:188-197

Holtz G, Johnson TR, Schrock ME: Paraneoplastic hypercalcemia in ovarian tumors. Obstet Gynecol 1979 Oct; 54:483-487

Patsner B, Piver MS, Lele SB, et al: Small cell carcinoma of the ovary: A rapidly lethal tumor occurring in the young. Gynecol Oncol 1985 Oct; 22:233-239

Young RH, Dickersin GR, Scully RE: Juvenile granulosa cell tumor of the ovary—A clinicopathological analysis of 125 cases. Am J Surg Pathol 1984 Aug; 8:575-596

Young RH, Scully RE: Ovarian sex cord stromal and steroid cell tumors, *In* Roth L, Czernobilsky B (Eds): Tumors and Tumorlike Conditions of the Ovary. New York, Churchill Livingstone, 1985, pp 43-73

Immunohistology for the Rapid Diagnosis of Viral Infections

THE DEVELOPMENT of increasingly aggressive and successful cancer therapy, the proliferation of organ transplant programs and the appearance of the acquired immunodeficiency syndrome have combined to place large numbers of immunocompromised patients at risk for potentially lethal viral infections. Fortunately, effective antiviral agents are becoming available. Their successful use, however, requires rapid and specific diagnosis.

Seroconversion is slow, intrinsically undependable in these patients and possibly irrelevant to the immediate clinical problem. Routine cultures are also slow and may merely reflect viral shedding rather than significant tissue involvement.

Pathologists' approach to the diagnosis of viral infection has traditionally been to identify characteristic cellular cytopathic effects, predominantly viral inclusions and associated alterations such as cellular enlargement, multinucleation and chromatin margination. This approach is valuable but does have disadvantages. False-negative results reversed by subsequent culture isolation may be distressingly frequent, especially with cytomegalovirus (CMV). Diagnostic cellular changes are probably absent in early infections in which the institution of therapy may be most beneficial. It is clear that viral nucleic acid and antigens are present in many cells that lack inclusions. In contrast, reactive alterations such as cellular enlargement and prominent nucleoli may produce false-positive results. Even when present, viral features may be recognizable without allowing specific virus identification, an increasingly important problem as effective antiviral agents with a narrow spectrum of activity are developed. For instance, acyclovir is eight to ten times less effective against varicella zoster virus (VZV) than against herpes simplex virus (HSV). Unfortunately, these two viruses cannot be distinguished on the basis of a Tzanck preparation or routine histologic or electron-microscopic examination.

Rapid direct identification of specific viruses through immunologic staining of tissue sections and smears has been hampered by limited antibody availability, though it has been feasible for many years. Monoclonal antibodies, some commercially available, are now making direct viral diagnosis practical. For example, we have found that the sensitivity of antibody C5 (Genetic Systems, Inc, Seattle/Syva Company, Palo Alto, California) in detecting CMV in frozen sections from open-lung biopsy specimens is essentially equal to that of culture or in situ nucleic acid hybridization. Other antibodies from these companies have been extremely valuable in the direct rapid diagnosis of VZV and HSV infections because they recognize glycoprotein antigens that withstand formalin fixation and paraffin embedding. A monoclonal antibody to the adenovirus hexon protein (C. Cepko and P. Sharp, Massachusetts Institute of Technology, Cambridge) and a polyclonal antibody to respiratory syncytial virus (Burroughs Wellcome Company, Research Triangle Park, NC) have also performed well. So many laboratories are now developing antibodies that soon all major viral pathogens will probably be directly detectable in tissue, even following routine fixation and processing.

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REFERENCES

Hackman RC, Myerson D, Meyers JD, et al: Rapid diagnosis of cytomegaloviral pneumonia by tissue immunofluorescence with a murine monoclonal antibody. J Infect Dis 1985 Feb; 151:325-329

Myerson D, Hackman RC, Meyers JD: Diagnosis of cytomegaloviral pneumonia by in situ hybridization. J Infect Dis 1984 Aug; 150:272-277

Shields AF, Hackman RC, Fife KH, et al: Adenovirus infections in patients undergoing bone-marrow transplantation. N Engl J Med 1985 Feb; 312:529-533

Chlamydia trachomatis—Diagnostic Techniques

Chlamydia trachomatis is a fastidious obligate intracellular parasite. The organism has been implicated as the causative agent in a variety of genital infections—including cervicitis, salpingitis, prostatitis and epididymitis—in upper respiratory tract infections and in conjunctivitis in infants. Chlamydia is effectively treated with antibiotics, but clinical manifestations may be nonspecific and subtle, and the organism has been difficult to detect.

Culture yields the highest detection rate with about 80% sensitivity, but culture is tedious and delays initiation of therapy. Special techniques are needed, making culture costly and not readily available in most laboratories.

Cytologic examination of secretions has proved to be an insensitive procedure for detecting *Chlamydia.* Cellular changes are generally nonspecific, leading to both false-positive and false-negative results. Adding iodine staining does not appreciably improve detection rates. Immunoperoxidase staining has recently been coupled with cytology, and results with this technique have varied. Technical difficulties with sample size, cell loss and nonspecific staining have precluded its widespread use.

Microimmunofluorescence for detecting antibody is both sensitive and specific for the organism. Special techniques, however, are required for its performance, and, like culture, microimmunofluorescence has not been available in most laboratories.

Direct fluorescent antibody staining for *Chlamydia* using fluorescein-conjugated monoclonal antibody to detect extracellular elementary bodies is probably the most promising procedure to date. The procedure can be used directly on smeared secretions or to enhance detection of the organism in culture and is both sensitive and specific for the organism. While not yet universally available, the procedure does not require sophisticated technical expertise and is inexpensive to do, making it more attractive to most laboratories.

Chlamydia trachomatis is difficult to detect. Direct fluorescent antibody staining appears to be the most promising, rapid and cost-effective technique at present, but, for any procedure to be effective, a heightened clinical awareness of the organism in high-risk populations is needed.

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REFERENCES

Geerling S, Nettum JA, Lindner LE, et al: Sensitivity and specificity of the Papanicolaou-stained cervical smear in the diagnosis of *Chlamydia trachomatis* infection. Acta Cytol 1985 Sep-Oct; 29:671-675

Schachter J, Dawson CR: Human Chlamydia Infections. Littleton, Mass, PSG Publishing, 1978

Tam MR, Stamm WD, Handsfield HH, et al: Culture-independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. N Engl J Med 1984 May; 310:1146-1150

Lymphoma—Correlating Morphology and Clinical Course of Disease

MAJOR CONCEPTUAL ADVANCES have recently been made regarding non-Hodgkin's lymphomas. These advances relate to a greater understanding of the immune system and the normal mechanisms of lymphocyte function. With this newly acquired knowledge, alternative classification systems for the malignant lymphomas have been proposed, based on modern immunologic concepts. New diseases have been described, and new approaches to the management of patients with lymphoma have been suggested. About 70% to 75% of lymphomas are of B-lymphoid origin, most arising from the cells that normally compose the follicular or germinal center of the node. About 20% to 30% of lymphomas are of T-lymphoid origin. In an attempt to correlate these concepts with clinical behavior, a translational tool was recently proposed, termed the "Working Formulation for Clinical Usage." In this system, lymphomas are divided into low-, intermediate- or high-grade tumors.

The low-grade lymphomas consist primarily of the follicular small-cleaved tumor (also known in the Rappaport system as nodular, poorly differentiated lymphocytic) and the lymphomas of small lymphocytes (also known as chronic lymphocytic leukemia, or well-differentiated lymphocytic lymphoma). These tumors are clinically indolent in behavior, with prolonged patient survival in spite of widely disseminated disease. Using multiagent chemotherapy, the group from Stanford has found that patients with low-grade lymphomas are not curable in the traditional sense, although they may be expected to live for five to ten years or more in the presence of active underlying lymphomatous disease. While in unusual cases a patient with localized (pathologic stage I) disease may be curable with radiation therapy, chemotherapy, consisting of single-agent alkylators or the cyclophosphamide-vincristine-prednisone regimen, is reserved for patients whose disease has become symptomatic or anatomically precarious.

The concept of prolonged survival in the presence of active disease is not valid in the lymphomas of intermediate- or high-grade type. These tumors, by natural history, are more aggressive and require intensive therapy at the time of diagnosis. Without the benefit of therapy, such patients would be expected to live less than a year. Newer regimens of multiagent chemotherapy have recently been described, however, that may result in long-term disease-free survival and possibly cure. These regimens, including the M-BACOD (methotrexate alternating with a combination of bleomycin, doxorubicin [Adriamycin], cyclophosphamide, vincristine [Oncovin] and dexamethasone) or similar therapies, have resulted in complete remission in about 60% to 70% of patients, with long-term, disease-free survival in about 50% of all treated patients. The specific details regarding optimal therapy in these patients depend on the specific subtype of disease, extent of disease and presence or absence of various prognostic indicators.

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REFERENCES

Levine AM: Non-Hodgkin's lymphomas: Clinical features. Semin Ultrasound CT MR 1985 Dec; 6:374-379

National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: Summary and description of a working formulation for clinical usage. Cancer 1982; 49:2112-2135

Portlock CS: 'Good risk' non-Hodgkin's lymphomas: Approaches to management. Semin Hematol 1983; 20:25-34

Skarin AT, Canellos GP, Rosenthal DS, et al: Improved prognosis of diffuse histiocytic and undifferentiated lymphoma by use of high dose methotrexate alternating with standard agents (M-BACOD). J Clin Oncol 1983 Feb; 1:91-98

Rectal Suction Biopsy in the Diagnosis of Congenital Megacolon

WHEN WE THINK of procedures used for either ruling in or ruling out the diagnosis of congenital megacolon (Hirschsprung's disease), biopsy of the muscularis propria of the rectum comes to mind as the procedure of choice. While muscularis biopsy is undoubtedly highly accurate, there are several drawbacks. It requires admission to hospital and general anesthesia. The operation may result in inflammation and scarring that make subsequent pullthrough operations difficult. The frozen tissue section, on which a pathologist is asked to make a pronouncement as to the presence or absence of ganglion cells, is generally of a poorer technical quality than well-fixed paraffin-embedded permanent sections. As a result, immature ganglion cells may not be recognized.

An alternative to muscularis biopsy is mucosal suction biopsy. The procedure avoids the disadvantages of muscularis biopsy in that anesthesia is unnecessary and the rectal wall is not incised. Only small fragments of mucosa and submucosa are obtained, which are fixed, embedded and stained in the routine manner.

The rationale behind this procedure is based on the fact that the submucosal (Meissner's) plexus bears a constant relationship to the myenteric (Auerbach's) plexus. During fetal development the submucosal plexus is populated by primitive neural elements that migrate from the underlying myenteric plexus. Therefore, the presence of ganglion cells in the submucosal layer assures their presence in the myenteric plexus. Their (ganglion cells) presence, no matter how immature, rules out Hirschsprung's disease.

The diagnostic accuracy of this procedure depends on the recognition of the characteristic morphology of the neural units composing the submucosal plexus and the appearance of ganglion cells in all their stages of maturity. Accuracy also depends on the adequacy of the sample obtained. There are some drawbacks to the method relating mostly to the size of the sample obtained and the failure to recognize immature ganglion cells. In my experience, however (more than 60 rectal biopsies), and that of others, the procedure has been highly accurate in determining the presence, or absence, of rectal ganglion cells. The technique may be further enhanced